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(54) Title: IMMUNOMODULATING GLYCOPEPTIDE

(57) Abstract

A product which causes super-proliferation of T cells for use in treating or diagnosing a disease, in particular cancer. In one embodiment the product is a glycopeptide with amino acid sequence from the MUC1 tandem repeat. The product may be used to treat cells *in vitro* or as an adjuvant.

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IMMUNOMODULATING GLYCOPEPTIDE

The invention relates to a product which causes super-proliferation of T cells for use in a method of treatment or diagnosis; an assay to identify such a product; an *ex-vivo* cell treated with the product and a vaccine comprising the product and/or the 5 *ex-vivo* cell.

The immune system is able to respond to infection by a pathogen or to the development of cancer in an antigen-specific manner. Vaccination with appropriate antigens can thus be used to prevent or treat infections and cancer. However administration of the antigen alone to an individual is often insufficient to cause the 10 development of an effective immune response to the antigen. This is particularly true if the infection or cancer causes immunosuppression. Adjuvants are therefore administered with the antigen to increase the magnitude of the immune response produced.

Another method of treating an infection or cancer is adoptive immunotherapy. 15 In this method T cells produced *in vitro* and specific to the required antigen are administered to the patient. Generally this is done by taking T cells from the patient, increasing their number by replication and administering them to the patient. However, replication of T cells *in vitro* to the required numbers is difficult.

The inventors have demonstrated that T cells (even antigen naive T cells) can 20 be stimulated to proliferate to an unusually large degree. This phenomenon is termed 'super-proliferation' herein. A method (described below) for identifying materials which cause such a T cell response has been developed. The effects of certain products identified in this manner on T cells responses *in vitro* has been investigated.

Products which cause super-proliferation of T cells can be used as vaccine 25 adjuvants or to replicate T cells to high numbers *in vitro*. The products can also be used to produce immune responses which have a specificity towards the product itself, as well as in the detection of T cell responses which may or may not be directed to the product.

The invention thus provides a product, which product causes super- 30 proliferation of T cells, for use in a method of treatment of the human or animal body

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by therapy or for use in a diagnostic method practised on the human or animal body.

The invention also provides a use of the product to treat a cell *in vitro*. The invention provides an *ex-vivo* cell treated with the product. The invention additionally provides a vaccine comprising an antigenic component and, as an 5 adjuvant a product of the invention.

In one embodiment the product comprises an antigen which is present on the surface of cancer cells, or the product is capable of stimulating T cells which are specific for cancer cells. Thus the invention also provides a use of the product in the manufacture of a medicament to prevent or treat cancer.

10 The invention is illustrated by the accompanying drawings in which:

Figure 1 shows FACS analysis of A2 stimulated cells. A is control, B is CD19 and C is CD3, CD16/56. CD19 is a B cell marker, CD16 and CD56 are NK markers and CD3 is a T cell marker.

Figure 2 shows FACS analyses of cells that proliferate after stimulation with 15 A2 GalNac. A is the control, B is anti-CD4, C is anti-CD8, D is anti- α/β TCR and E is anti- γ/δ TCR. The vertical axes show counts.

A product of the invention is generally an individual compound, but can be a composition comprising one or more active compounds which each contribute to causing super-proliferation. The product typically comprises at least 5 hydroxyl 20 groups, such as at least 10, 20 or 30 hydroxyl groups. The product typically comprises one or more of the modifications which may be present on the peptide discussed below.

Certain products of the invention are able to bind to one or more different MHC class I or class II molecules. In one embodiment the product is able to bind to a 25 receptor on an antigen presenting cell or a T cell, such as a receptor which can bind to glycopeptides. The C-type lectin of macrophages is an example of such a receptor (for example as described in reference 1). After binding to the receptor the product can typically be taken up by the antigen presenting cell and be presented on the surface of the cell bound to a MHC class I or class II molecule.

30 Certain products of the invention are peptides or comprise a peptide. The

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peptide may comprise sequence from a naturally occurring protein, such as from a human, animal or pathogen, and may thus be a fragment of such a protein. The protein is typically one which is glycosylated in nature. In one embodiment the protein is one which occurs in a different form in tumour cells, such as having a 5 different pattern of glycosylation in tumour cells. The protein is typically one which is expressed on the surface of cells.

In one embodiment the protein is MUC1. Thus the peptide may comprise amino acid sequence from the repeat sequence of MUC1, such as the sequence N-Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-10 Pro-Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-C (SEQ ID NO:2), or a fragment of this sequence. A preferred fragment is N-Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-C (SEQ ID NO:1).

The sequence of the peptide may have homology with any of the peptides 15 mentioned above, such as at least 70% homology, preferably at least 80, 90, 95, 97 or 99% homology, for example over a region of at least 20, preferably at least 30, for instance at least 40, 60 or 100 or more contiguous amino acids. Methods of measuring protein homology are well known in the art and it will be understood by those of skill in the art that in the present context, homology is calculated on the 20 basis of amino acid identity (sometimes referred to as "hard homology").

For example the UWGCG Package (2) provides the BESTFIT program which can be used to calculate homology (for example used on its default settings). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul 25 S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pair (HSPs) by 30 identifying short words of length W in the query sequence that either match or satisfy

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- some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, *supra*). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both 5 directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached.
- 10 The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.
- 15 The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would 20 occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.
- The homologous peptide typically differs from the original sequence by 25 substitution, insertion or deletion, for example from 1, 2, 3, 4, 5 to 8 or more substitutions, deletions or insertions. The substitutions are preferably 'conservative'. These are defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

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ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
Polar - charged	D E	
	K R	
AROMATIC		H F W Y

A polynucleotide sequence encoding the homologous peptide typically

- 5 hybridises with a polynucleotide encoding the original peptide. It typically hybridises at a level significantly above background. The signal level generated by the interaction is typically at least 10 fold, preferably at least 100 fold, as intense as 'background' hybridisation. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ^{32}P . Selective hybridisation is
- 10 typically achieved using conditions of medium to high stringency (for example 0.03M sodium chloride and 0.003M sodium citrate at from about 50°C to about 60°C).

The peptide (or fragments referred to above) generally has a length of at least 15 amino acids, such as at least 20, 21, 22, 25, 30, 40, 60 or 80 amino acids. The 15 peptide is typically modified, such as by a naturally occurring or non-naturally occurring modification. The modification may be glycosylation, such as by the addition of 1, 2, 3, 4 or more carbohydrate moieties to the peptide, for example to different amino acids of the peptide. The carbohydrate moieties typically comprise natural and/or non-natural sugars which may be present in the form of 20 monosaccharides, disaccharides, trisaccharides or polysaccharides. The sugars present in the moieties may be aldoses and/or ketoses. The carbohydrate moiety generally comprises a sugar which is a tetrose, pentose, hexose or heptose. Typically the sugar is Glu, Gal or GalNac. A preferred moiety is Gal-GalNac (Gal β 1-3GalNac α). If the peptide comprises amino acid sequence from a naturally occurring

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protein it may also comprise one or more modifications in that sequence which are present in the naturally occurring forms of the protein, such as the patterns of glycosylation. The peptide typically comprises less glycosylation than present in the equivalent sequence in the naturally occurring protein, such as less than 80%, 50% or 5 30% of the number of sugars present in the naturally occurring sequence. The modification(s) present in the peptide is typically the same as when the protein is present in a non-tumour cell or a tumour cell.

The carbohydrate moiety is generally attached to a serine, threonine or asparagine residue in the peptide, and is typically O-linked or N-linked to the 10 peptide. The moiety is generally attached to the peptide through an α or β linkage.

When the peptide has the sequence of SEQ ID NO:1 or a homologous sequence the modification is typically present on positions 10 and/or 17, (i.e. the tenth and seventeenth residues respectively from the N terminal of the peptide), or the equivalent positions in a homologous peptide. A preferred product comprises a 15 glycopeptide with the amino acid sequence of SEQ ID NO:1 which has a Gal-GalNac (Gal β 1-3GalNac α) or GalNac (N-acetyl galactosamine α) moiety on Thr at position 10 or Thr at position 17. This glycopeptide is referred to as the '10, 17 glycopeptide' herein. Thus in a preferred embodiment the product is peptide A2, A2 GalNac or A3 as shown in Table 2.

20 Certain products are able to bind to a specific binding agent of the 10, 17 glycopeptide. Such a specific binding agent may be the receptor on T cells which the 10, 17 glycopeptide binds to in order to cause super-proliferation in the assay described below. The specific binding agent may be an antibody specific to the 10, 17 glycopeptide. Such an antibody can be produced by routine methods, such as 25 those described below. The specific binding agent will generally be able to inhibit super-proliferation caused by the 10, 17 glycopeptide in the assay described below. Products able to bind to a specific binding agent of the 10, 17 glycopeptide can generally compete with the 10, 17 glycopeptide in binding to the specific binding agent or to T cells, and therefore will inhibit the binding of the 10, 17 glycopeptide to 30 the specific binding agent or to T cells.

The product of the invention may or may not include peptides comprising sequence from the tandem repeat of MUC1 (such as SEQ ID NO:1 or SEQ ID NO:2; or a fragment thereof) which have GalNac or GalNac-sialic acid attached to (i) the Thr of the Gly-Val-Thr-Ser-Ala motif, or (ii) the Ser and/or Thr of the Gly-Ser-Thr-Ala-Pro motif.

A product according to the invention which causes super-proliferation of T cells is one which gives a stimulation index of at least 10 or more when used in the assay described below. The product may thus give a stimulation index of at least 15, 20, 30, 50, 80, 100, 200 or more in this assay.

10

Super-proliferation assay

Peripheral blood lymphocytes (PBL) are prepared from 20ml of blood from a healthy human donor. The buffy coat containing the PBL is separated from the other blood components by centrifuging using a Ficoll gradient. PBL separated in this way will contain antigen presenting cells. 2 x 10⁵ PBL are added to a well of a 96 well plate in 200µl of AIM V medium and 50µg/ml of the candidate substance (the AIM V medium used is typically Cat. No. 12030-011 from GIBCO BRL). A control well is set up in the same way but does not contain the candidate substance. The well is incubated for 96 hours at 37°C. 1µCi of ³H thymidine is then added to the well and a further incubation for 18 hours at 37°C is performed. The cells are harvested and the incorporation of thymidine is determined. The stimulation index is calculated by dividing the average counts obtained for the candidate substance by the average counts obtained for the cells in the control well.

In the case of certain products the super-proliferation is inhibited by antibodies which bind to MHC class I molecules and/or antibodies which bind to MHC class II molecules, such as W632 antibody which binds class I molecules or Tal 3c3 which binds class II molecules.

The product typically causes super-proliferation of any of the T cells described below which can be treated with the product, for example at a concentration of 50µg/ml.

Products according to the invention cause super-proliferation in the assay described above. They may or may not cause super-proliferation of T cells *in vivo* or of T cells which are different to the T cells used in the assay. However, in general, the products of the invention will cause super-proliferation of other T cells or super-proliferation of T cells in conditions other than those of the assay.

The inventors have shown that when rodent cells (such as mouse or rat cells) are treated with certain products of the invention these cells do not undergo super-proliferation. Thus the product may or may not be able to cause super-proliferation of T cells of rodents. Thus if products which do not cause super-proliferation of 10 rodent T cells are used (with rodent T cells) in the assay described above then generally the stimulation index will be less than 10, for example less than 8, 6, 4, 2 or 1.

The product of the invention may be produced by conventional techniques well known to those skilled in the relevant arts. Peptides may be synthesised *de novo* 15 or produced by transcription and translation of DNA or translation of RNA in a suitable expression system by conventional methods. Products which are new chemical compounds may be produced by known techniques of synthetic organic chemistry. Methods of making glycopeptides are known in the art (3). Where the product is or comprises a glycopeptide the carbohydrate side-chains can typically be 20 added to the glycopeptide using the relevant glycosyl transferase(s). Typically the carbohydrate side-chain is built up on the peptide by step-wise addition of saccharide units. For example, if the saccharide unit which is directly attached to the peptide is GalNac then this can be added to a threonine or serine in the peptide by providing UDP-GalNac and the peptide to polypeptide-GalNac-transferase.

In this specification the term 'stimulation' refers to causing the T cell to become activated after the contact of a receptor on the surface of the T cell with the product. The activation may include the differentiation and/or proliferation of the T cell. The activation may cause the T cell to begin to secrete or increase its secretion of cytokines. The stimulation may be antigen specific or non-specific. When it is 30 non-specific different T-cells carrying T-cell receptors specific to different antigens

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will be stimulated to proliferate. Thus the stimulated T-cells may be monoclonal or polyclonal.

Certain products are presented to the T cell on a MHC class I or II molecule. The stimulation may be specific or non-specific with regards to the MHC class I or II molecules which present the product to the T cell. The proliferation which occurs upon stimulation may be super-proliferation.

The stimulation may be of primary (antigen naive) T cells or secondary (antigen experienced) T cells. Generally primary T cells have not been previously exposed to the antigen which is recognised by the T cell receptor which they carry.

10 Secondary T cells are formed from primary T cells by replication of the primary T cell in response to exposure to an antigen which is recognised by the T cell receptor. The T cells will be CD4 or CD8 positive. In one embodiment the T cell will recognise the product in a MHC class II or class I restricted manner. The CD4 T cell may be a Th1 or Th2 type cell, Th1 being preferred. The T cell may be a cytotoxic T

15 cell.

The T cell may be a lymphokine-activated killer (LAK) cell. The production of LAK cells is known in the art (8). These cells are generally produced by treating peripheral blood lymphocytes with IL-2. The T cell may be a tumour infiltrating lymphocyte (TIL). TIL can be produced by culturing a dissociated tumour in IL-2

20 and then separating the differentiated lymphoblasts which are produced. One method of producing TIL is described below. The T cell may be derived from a lymph node which drains a tumour.

The T cells may carry a T cell receptor which is specific to an antigen from a pathogen, such as a virus, bacterium or fungus. The T cell receptor may be specific to

25 a cancer antigen, for example an antigen which is produced by cells of any of the cancers mentioned below.

The invention provides the use of the product to treat a cell *in vitro*. The cell is treated in a method comprising contacting the cell with the product. Typically in the method the product is present at a concentration of at least 0.1 μ g/ml, such as (e.g.

30 at least or about) 1 μ g/ml, 10 μ g/ml, 50 μ g/ml, 100 μ g/ml, 1000 μ g/ml or more. The

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cell is typically contacted with the product for at least 0.5 hours, such as (e.g. at least or about) 2, 10, 24, 48, 96 or more hours under conditions in which the cells remain alive (and typically under conditions allowing the cells to remain functionally active) so that the cell is generally able to interact with the product in accordance with its
5 natural function, for example the cell may be stimulated by the product or may present the product on its surface. The cell which is treated is generally a T cell as described above or an antigen presenting cell.

The product may be used to treat a population of cells comprising T cells and antigen presenting cells (for example present in the amounts described below for
10 composition of the invention). The cells which are to be treated are generally derived from a human or animal. The cells may be from an individual who is healthy, who may or may not be at risk from a disease. The individual may have a disease. In the case where the individual has a tumour the cells may be derived from the blood or from the vicinity of a tumour, or may be cells which have infiltrated a tumour. The
15 cells may be from the lymphatic system, such as from a lymph node. The lymph node may be one which receives lymph fluid from a tumour.

In the case of T cells the product will generally cause stimulation of the T cells. As noted above the stimulation may cause super-proliferation, and thus the invention provides a method of causing super-proliferation of a T cell comprising
20 contacting the product with a T cell. Generally antigen presenting cells will also be present during stimulation of T cells, and these may present the product to the T cell, for example bound to a MHC class I or II molecule. Certain products are capable of stimulating the T cells in the absence of antigen presenting cells.

In the method of the invention the T cells may be additionally treated with
25 other agents. These other agents may enhance the stimulation caused by the product or may cause only specific T cells to be stimulated. Such agents include cytokines, such as IL-2 or IL-7; adjuvants, such as keyhole limpet cyanin or any of the other adjuvants mentioned herein; or antigens which are recognised by the T cells. As noted above generally during the T cell stimulation antigen presenting cells will be
30 present. However, the agents may also include other cells, such as CD4 T cells in the

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case where CD8 T cells are being stimulated.

In the method the stimulation of the T cells may be under conditions where the T cells proliferate in response to the stimulation. Thus the method can be used to 'grow up' (i.e. increase the numbers of) T cells which have been taken from an
5 individual.

As discussed above the T cells which are stimulated may be primary T cells, and thus the method of the invention can be used to produce a primary T cell response *in vitro*, i.e. to cause proliferation of primary T cells to the extent that they are detectable in proliferation or cytotoxic assays. Reference 9 shows for example
10 how such assays may be performed.

Since the stimulation of the T cells in the method of the invention generally causes proliferation of the T cells the method may be used to detect particular T cells, such as T cells specific for a particular antigen. Generally in the detection of T cells specific to a particular antigen proliferation of the T cells, secretion of substances
15 from the T cell or killing of target cells by the T cell; in response to the antigen is used as the basis of a detection assay. However such assays are limited in their sensitivity of detection of the T cells and are unable to detect T cells present at low concentrations. Thus the product of the invention can be used to cause proliferation of such T cells to aid their detection in such assays. The antigen which the T cells
20 recognise may be the product itself.

Thus the method of the invention can be used to detect T cells specific to the product. The method may also be used to detect T cells which are not specific for the product. In such a method the antigen which the T cells are specific for will also be provided to the T cells. If a T cell response specific to the product is produced during
25 a particular disease then the method of the invention may be used to detect this T cell response and thus can be used in the diagnosis of the disease. In particular such a disease may be one which causes immunosuppression. The disease may be caused by a pathogen or a tumour, such as any of the cancers discussed below.

Generally the T cells taken from an individual with a disease which are to be
30 stimulated in the method will be secondary T cells. These T cells will be specific for

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antigens produced by the entity causing the disease. Such an entity will generally be a pathogen or a tumour.

The antigen presenting cell which is treated in the method of the invention may be a cell which naturally acts as a professional antigen presenting cell or a may 5 be non-natural antigen presenting cell which is able to present the product to the T cell. The antigen presenting cell is typically a dendritic cell, monocyte, macrophage or B cell. The antigen presenting cell may be the Drosophila cell described in WO 93/17095 which is capable of antigen presentation, CHO cells, baculovirus infected insect cells, bacteria, yeast or vaccinia-infected cells. The antigen presentation 10 system may be based on (10). In one embodiment the dendritic cell has been cultured *ex vivo* from CD34+ stem cells or CD14+ peripheral blood monocytes, for example using GM-CSF, IL-4 and TNF- α . The antigen presenting cell may be treated with the product in order to present the product in a suitable context to the T cell, such as bound to MHC class I or class II molecules. Thus the treatment of the antigen 15 presenting cell generally makes it capable of stimulating a T cell able to recognise the antigen.

The cell which is treated may be a cell which presents empty MHC class I or II molecules on its surface. Such a cell may be defective in MHC presentation. The cell which is treated may be a T2 cell.

20 The *ex-vivo* cell of the invention is generally a T cell which has been stimulated by the product or an antigen presenting cell which has been provided with the product. Such cells can be made in the method of the invention. The stimulation of the T cell may or may not be antigen specific, and therefore the T cell may or may not have a T cell receptor which recognises the product. The T cell may be present in 25 a polyclonal or monoclonal population of T cells. The T cell of the invention may have any of the characteristics of the T cells described above.

Generally the antigen presenting cell of the invention carries the product on its surface in conjunction with an MHC class I or class II molecule. In one embodiment the antigen presenting cell has at least 200, for example at least or about 30 500 or 1000, class I or class II molecules on its surface loaded with the product.

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The cells of the invention may be labelled or may carry a label, such as labelled thymidine or radioactive chromium.

The invention also provides a composition comprising T cells, antigen presenting cells and a product of the invention. The T cells or antigen presenting cells 5 may be any of the cells mentioned above. The T cell:antigen presenting cell ratio is typically from 500:1 to 1:500. Typically at least 10^3 , such as (e.g. at least or about) 10^5 , 10^6 , 10^7 , 10^8 , 10^9 cells are present per millilitre of the composition. The composition typically also comprises a culture medium capable of supporting the T cells or antigen presenting cells, such as RPMI medium. The medium may also 10 comprise cytokines, such as IL-2, IL-4, IL-7 or TNF- α . The T cells and antigen presenting cells may be from the same individual. The composition may comprise a mononuclear cell fraction, for example which has been separated from peripheral blood.

In the vaccine of the invention the product may act as an antigen and/or as an 15 adjuvant. Generally the product acts as an antigen if the vaccine leads to the stimulation of T cells which carry a receptor specific for the product. The product acts as an adjuvant if the vaccine leads to the stimulation of T cells which do not carry a T cell receptor which is specific for the product. The product may act as an antigen or adjuvant in a manner that is dependent on or independent of the MHC 20 class I or II type of the individual to whom the vaccine is administered.

In a vaccine where the product is used as an adjuvant the antigenic component may be from a pathogen or a cancer, such as the pathogens or cancers discussed herein.

In a vaccine where the product is used as an antigen the adjuvant component 25 may be one which favours the development of a T cell response (for example a CD4 or CD8 T cell response) over an antibody response, such as of any of the T cells discussed above. Thus the adjuvant component may comprise a cytokine such as IL-2 or IL-7, keyhole limpet hemocyanin, tetanus toxin or LAMPI (e.g. as described in (12)). The adjuvant may comprise an oil droplet emulsion, squalene, or components 30 of bacteria, such as Mycobacterium phlei (e.g. cell wall skeleton) or Salmonella

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minnesota (e.g. menophosphoryl lipid A). The adjuvant may be DetoxTM.

In the embodiment the adjuvant is a liposome, for example as in (11). The product may be linked to a substance which directs it to the MHC class I or II pathway, such as a tat peptide, for example as in (13).

5 When the vaccine is administered to a human or animal an antibody response may or may not occur. The antibodies may be specific for the product. When the vaccine contains an antigenic component which is not the product the antibodies may be specific for such a component.

The cells of the invention may also be used in a method of treatment of the
10 human or animal body by therapy. The cells may be administered to a patient. The cells which are administered may be autologous cells, or cells which have been partially or fully matched with the patient for MHC class I HLA-A or HLA-B; or for MHC class II type. The cells may be present in or derived from the composition of the invention discussed above. The cells may be separated from the composition
15 using a specific binding agent which binds either the cell to be administered to the patient or the other cells in the composition which need to be removed. Typically the specific binding agent is an antibody.

The invention also provides an assay for identifying a product which causes super-proliferation of T cells comprising contacting a compound or composition with
20 a T cell and determining whether the T cell undergoes super-proliferation. The assay is typically the assay described above. The assay may thus be used to screen candidate substances. Such candidate substances may be present in extracts from human or animal cells, such as extracts from tumour cells. The candidate substances may be present in combinatorial libraries or natural product libraries, such as phage
25 display libraries. The assay may also be used to design products of the invention.

Thus the invention provides a product which causes super-proliferation of T cells and which is not described in a database at the date of filing of the application. Such a product is thus not known in the art at the date filing of the application.

The product of the invention may be provided in a kit for causing super-
30 proliferation of T cells. Such a kit may be for replicating cells *in vitro* prior to

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administering them to a patient (as described above for the method of causing super-proliferation of a T cell). The kit may be for diagnosing cancer, in which case the product is used to detect the presence of cancer specific T cells (such as T cells specific for the product), for example as described above for the detection of T cell responses.

The product or cell of the invention may be present in a substantially isolated form. It will be understood that the product or cell may be mixed with carriers or diluents which will not interfere with the intended purpose of the product or cell and still be regarded as substantially isolated. The product may be in substantially purified form in which case it will generally comprise more than 90%, (e.g. more than, at least or about) 95, 98 or 99% of dry mass in a preparation; or if the product is a peptide it may comprise more than 90%, (e.g. more than, at least or about) 95, 98 or 99% of the peptide in the preparation. The cell may be in a substantially purified form in which case it will generally comprise more than 90%, (e.g. more than, at least or about) 95, 98, or 99% of the cells or dry mass in the preparation.

The invention also provides a method of diagnosing or treating a disease by administering an effective non-toxic amount of a product or cell of the invention to a human or non-human animal in need thereof. The disease may be cancer, and thus the method may diagnose or treat carcinomas, for example mammary carcinoma, lung carcinoma, bladder carcinoma, colon carcinoma, ovary and endometrial tumours. Other cancers which may be diagnosed or treated include sarcomas, such as soft tissue and bone sarcomas, and haematological malignancies, such as leukemias.

The product or cell of the invention may be formulated for clinical administration by mixing them a pharmaceutically acceptable carrier or diluent. For example the product or cell may be formulated for topical, parenteral, intravenous, intramuscular, subcutaneous or transdermal administration. They may be mixed with any vehicle, for example a diluent or carrier which is pharmaceutically acceptable and appropriate for the desired route of administration. The pharmaceutical carrier or diluent for injection may be, for example, a sterile or isotonic solution such as Water for Injection or physiological saline.

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The dose may be adjusted to deliver an effective non-toxic amount and according to various parameters, especially according to the nature and efficacy of the substance used; the age, weight and condition of the patient to be treated; the mode of administration used; the conditions to be treated; and the required clinical 5 regimen. As a guide, the amount of the product of the invention to be administered by injection will generally be from 10 to 1000 μ g. For instance 100 to 500 μ g. As a guide the number of cells of the invention to be administered will generally be from 10^5 to 10^{13} , preferably from 10^7 to 10^{11} .

10 The routes of administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage for any particular patient and condition.

Method of administering cells are known in the art, for example as shown in US-A-4,844,893 and US-A-4,690,915.

15 Typically cells are administered in a solution with a volume of at least 20ml, for example at least 50ml, 100ml, 200ml or more. The cells can be given to the patient in 1, 2, 3, 10, 20 or more separate administrations, each administration separated, for example, by at least 1 hour, 2 hours, 5 hours, 12 hours, 1 day, 2 days, 4 days, 7 days, 1 month or more. Administration regimens for TIL are described in (4).

20 Methods of stimulating T cells in an antigen specific manner *in vitro* are known in the art, for example (5) shows how antigen-specific primary CD8 T cell responses can be produced.

Antibodies can be made to any of the substances mentioned herein, for example as described in (7). The antibodies may be purified or isolated, for example to any of the levels of purification discussed above for the product of the invention.

25

Preparation of TIL

Methods of producing TIL are known in the art, for example (6). TIL can be obtained by digesting a tumour in a collagenase, DNase type I and hyaluronidase type V enzyme mixture, filtering the resulting suspension (e.g. using a wire mesh), 30 and separating the lymphocytes from the filtrate using a Ficoll gradient. The TIL can

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be cultured at a density of 5×10^5 viable cells/ml in AIMV (or RPMI supplemented with 10% human serum), 6000 IU/ml of IL-2 and 1000U/ml of IL-4 at 37°C with 5% CO₂. When the TIL reach a density of 3 to 4×10^6 cells/ml the culture can be divided and diluted.

5

Substances which provide the product

Certain substances may provide the product of the invention when they are administered to an individual or when they are contacted with (e.g. provided inside) mammalian cells, e.g. any of the cells mentioned herein. Such substances are 10 included in the term 'product' used herein. Thus such substances can be used in the *in vivo* and *in vitro* methods discussed herein and can be administered in the same way as described for administration of the product.

The substance is typically a precursor of the product which can be processed (typically hydrolysed) by cells to provide the product. In the case of products which 15 are glycopeptides such processing may modify the peptide and/or carbohydrate component. The precursor may for example be capable of being processed in the antigen processing pathways of any of the antigen presenting cells mentioned herein. In the case of products which comprise peptides the precursor may comprise the product with additional sequence at the N and/or C terminal of the product.

20 In one embodiment the substance is a polynucleotide which is capable of being expressed to provide the product (or precursor discussed above). The polynucleotide is typically DNA or RNA, and is single or double stranded. If the product comprises a peptide, then the polynucleotide generally comprises sequence that encodes the peptide.

25 The coding sequence is typically operably linked to a control sequence capable of providing for expression of the polynucleotide. Thus typically the polynucleotide comprises 5' and 3' to the coding sequence sequences which aid expression, such as aiding transcription and/or translation of the coding sequence.

The polynucleotide is typically capable of expressing the product in a 30 mammalian cell, such as in a human cell. The polynucleotide may be capable of

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expressing the product in the cellular vector discussed below.

In one embodiment the polynucleotide is present in a virus or cellular vector, such as a virus which is capable of stimulating a CD8 T cell response, such as a vaccinia virus (e.g. MVA or NYVAC).

5 The following Examples illustrate the invention:

Example 1

Preparation of PBL

20-25ml of blood is collected into blood collection tubes (containing 20ml
10 RPMI, sodium citrate and mercaptoethanol) and layered onto Ficoll. The tubes are spun at 400g for 20 minutes and the buffy coat containing the PBLs removed and washed three times with Hanks buffered salt solution and once in AIMV medium. Antigen presenting cells are present in the preparation.

15 Preparation of dendritic cells (DCs)

20-25ml of blood is taken into blood collection tubes containing 20ml of RPMI, sodium citrate and mercaptoethanol and layered onto Ficoll. The tubes are spun at 400g for 20 minutes and the buffy coat containing the PBLs removed and washed three times with Hanks buffered salt solution (9) and once in AIMV medium.

20 Between 5 and 20 x 10⁶ cells are plated onto 33mm culture dishes in AIMV and cells allowed to adhere for 2 hours. The non-adherent cells are gently removed and the adherent cells cultured in AIMV medium containing 500 units/ml human IL-4 and 800 units/ml of human GM-CSF. 1ml of medium is removed every 2-3 days and replaced with fresh medium containing cytokines IL 4 and GM-CSF. On day 7
25 the dendritic cells are ready to be used as APCs.

Example 2

Proliferation assay in which glycopeptides are presented on the purified dendritic cells

30 Dendritic cells, cultured for 7 days as described above are harvested by

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pipetting and divided among an appropriate number of tubes (3 x 33mm dishes between 5 or so tubes, 15ml Falcons). The cells are spun and taken up in 1ml AIMV medium and incubated for 2 hours at 37°C with 20-100µg/ml of the glycopeptide or buffer alone. 2 x 10⁵ non-adherent PBLs (which were removed during purification of 5 the dendritic cells) in 100µl AIMV are mixed with 100µl of glycopeptide pulsed DCs in a well of a 96 well tissue culture dish in the presence of 5-50µg of glycopeptide. Each sample is done in quadruplicate and incubated for 4 days at 37°C. On day 4, 1µCi of 3H thymidine is added to each of the wells and the dish incubated overnight at 37°C. The cells are harvested and the incorporation of thymidine determined.

10

Proliferation assay in which the glycopeptides are added directly to PBL

2 x 10⁵ PBL are added to each well of a 96 well dish. Glycopeptides or buffer is added to the wells (each sample done in quadruplicate) and the dishes incubated at 37°C. On day 4, 1µCi of 3H thymidine is added to each of the wells and the dish 15 incubated overnight at 37°C. The cells are harvested and the incorporation of thymidine determined.

Calculation of stimulation indices

The stimulation index is calculated by dividing the average counts obtained 20 for the test glycopeptide by the average counts obtained for the cells with buffer alone. This is a standard way of presenting this type of data and it is generally thought that for a sample to be considered positive it must give a stimulation index of 3 or over. Results from proliferation assays are shown in Table 1. The glycopeptides tested are shown in Table 2 (they are linked to the peptide through an α linkage). A10 25 is the same as A9 but with a Lys at the N-terminal of the A9 sequence. Table 1 shows results with T cells from 7 healthy donors (left hand 7 columns) and 6 breast cancer patients (BrCa).

Example 3

30 Proliferation assay using CD4 T cells

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50 μ g/ml of glycopeptide was added to 2×10^5 CD4 T cells per well (in a flat bottomed 96 well plate).

		CPM \pm SD	SI
5	MEDIUM	4112 \pm 398	
	PHA	58987 \pm 4577	14.3
	A2	15912 \pm 2134	3.9
	A3	43744 \pm 6512	10.6
	A2GalNAc	48356 \pm 3671	11.8
10	A8	19182 \pm 4388	4.7
	A9	17823 \pm 5642	4.3
	A10	4816 \pm 765	1.2

Example 4

15 FACS analysis of cells that proliferate after stimulation with glycopeptide

PBL from a healthy donor were cultured with autologous DCs (pre-pulsed with 100 μ g/ml of A2 glycopeptide for 2.5 hours) in the presence of A2 glycopeptide for 7 days. IL-2 was then added to the cultures and after a further 7 days of culture the cells were again stimulated with A2 glycopeptide using autologous DCs. After a 20 further round of IL-2 addition and re-stimulation with the glycopeptide the cells were analysed by FACS. CD19 (B cell marker); CD16 and CD56 (NK markers); CD3 (T cell marker) specific markers were used. Results are shown in Figure 1.

PBL from a healthy donor were stimulated with A2GalNAc followed by IL-2 for 2 rounds of stimulation as described above for figure 1. Results of FACS 25 analysis are shown in Figure 2 in which: A, negative control; B, staining with anti-CD4 antibody; C staining with anti-CD8 antibody; D staining with anti- α/β T cell receptor antibody; E, staining with anti- γ/δ T cell receptor antibody.

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Example 5

Cytokine secretion by T cells after stimulation

The table below shows IFN α secretion by PBL in response to stimulation by the glycopeptides. Since there is no IL-4 secretion this is indicative of a TH₁ response.

Glycopeptide	Stimulation index	IFN γ secretion pg/ml	IL-4 secretion
No glycopeptide	1	0	0
A1	0.93	0	0
A2	9	400	0
A3	6	200	0
A4	10.9	860	0
A6GalNAc	5.5	330	0
A6	1.3	0	0
A8GalNAc	9	489	0

Example 6

Proliferation assays comparing mouse spleen cells to human PBL

Mouse spleen cells were used in a proliferation assay. The results are shown below. As can be seen even though A8GalNac causes super proliferation of human PBL it does not give a positive result with mouse spleen cells.

Proliferation Index		
	Mouse spleen cells	Human PBLs
Cells alone	1	1
PHA	8.3	-
A8	0.9	4.3
A8 GalNAc	2.5	18.7

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Proliferation of PBLs to glycopeptides. Result shown as stimulation indices

	Glyco peptide	Donors												
		JB	CW	IMc F	TP	DM	M	LS	BrC a 1	BrC a 2	BrC a 3	BrC a 4	BrC a 5	BrC a 6
5	A1	0.7	0.8		0.9, 1.5	1.7, 1.0	1.2	1.0			1.1	1.9	1.7	1.6
10	A1 Ga1NAc					1.6								
15	A2 GalNAc	5 29 20	20 29 20		22 9 3	24	8.8	24			9	29	48	30
20	A2		13 9 13								11	55	47	37
25	A3	20 87 11 5	13 45 43 6	17 14.5	9 10 5 7	47 28 18 21	8.8	27	6	15	8	32	33	33
	A4	2 21			4	17	10	24	2	5	10	31	35	32
	A5	1 5			1	4.4	1.4	4	2	3	1	9.5	10	31
	A6	1			5	34					3.7	21	23	32
	A7	5	14	5	2	6.4	2.2	4	2	1	1.2	3	3	13
	A8	5			1.5	7				1.4	1.8	2.5	4.4	
	A9				5.6	22				2	10	19	34	
	A10				1	2				1	1	1.4	1.7	
	MUC2 Ga1NAc	1	1		2	1.2	0.7	1.3			1.2	1.4	1.4	2.2
	MUC2										1.4	1.7	1.8	2.2
	gp120				1	1	1	1			1	1	1.7	1

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Table 2

5	A1			= GalNAc β 1-3Gal
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				
10	A2 GalNAc			= GalNAc
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				
15	A2			
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				
20	A3			
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				
25	A4			
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				
30	A5			
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				
35	A6			
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				
40	A7			
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				
45	A8			
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				
50	A9			
Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala				

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MUC2	<input type="checkbox"/>	
	<input checked="" type="checkbox"/>	
Thr-Thr-Thr-Val-Thr-Pro-Thr-Pro-Thr-Gly		
5		
MUC2 GalNAc	<input type="checkbox"/>	
	<input checked="" type="checkbox"/>	
Pro-Thr-Pro-Thr-Gly-Thr-Glu-Thr-Pro-Thr-Thr-Pro-Ile-Thr-Thr-Thr-Thr-Val-Thr-Pro-Thr		
10 gp120	<input type="checkbox"/>	
	<input checked="" type="checkbox"/>	
Arg-Ile-Arg-Ile-Gln-Arg-Arg-Gly-Pro-Gly-Arg-Ala-Phe-Val-Thr-Ile-Gly-Lys		

-25-

References

1. Suzuki *et al* (1996) *The Journal of Immunology*, **156**, 128-135.
- 5 2. Devereux *et al* (1984) *Nucleic Acids Research* **12**, 387-395.
3. Mathieu *et al* (1997) *J.Chem. Soc., Perkin Trans. 1*, 2359-68.
4. Ratto *et al* (1996) *Cancer*, **78**, 244-250.
- 10 5. Plebanski *et al* (1995) *Eur. J. Immunol.*, **25**, 1783-87.
6. Lotze (1992) *Human Gene Therapy*, **3**, 167-177.
- 15 7. Kohler and Milstein (1975) *Nature* **256**, 495-497.
8. Chang and Shu (1996) *Crit. Rev. in Oncol. Hematol.* **22**, 213-228.
9. Current Protocols in Immunology, Volume 2, edited by Coligan *et al*, John Wiley & Sons 1997.
- 20 10. Porta *et al* (1994) *Virology* **202**, 949-955.
11. Guan *et al* (1998) *Bioconj. Chem.* **9**, 451-458.
- 25 12. Wu *et al* (1995) *Proc. Natl. Acad. Sci.* **92**, 11671-11675.
13. Kim *et al* (1997) *J. Immunol.* **159**, 1666-1668.

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CLAIMS

1. A product which causes super-proliferation of T cells for use in a method of treatment of the human or animal body by therapy or for use in a diagnostic method practised on the human or animal body.
- 5 2. A product according to claim 1 which is a glycopeptide with the amino acid sequence of SEQ ID NO:1 which has a Gal-GalNac or GalNac moiety on Thr10 or Thr17, or an analogue thereof which causes super-proliferation of T cells for use in a method of treatment of the human or animal body by therapy or for use in a diagnostic method practised on the human or animal body.
- 10 3. A product which causes super-proliferation of T cells and which is not described in a database at the date of filing of the application.
4. An assay for identifying a product which causes super-proliferation of T cells comprising contacting a compound or composition with a T cell and determining whether the T cell undergoes super-proliferation.
- 15 5. Use of a product as defined in any of claims 1 to 3 or identified in the assay of claim 4 to treat a cell *in vitro*.
6. An *ex-vivo* T cell which has been treated with a product as defined in any one of claims 1 to 3 or identified in the assay of claim 4.
- 20 7. An *ex vivo* cell which has been treated with a product as defined in any one of claims 1 to 3 or identified in the assay of claim 4 and which is capable of presenting the product to a T cell.
8. A T cell according to claim 6 which is a cell that has undergone super-proliferation after being treated with the product.
- 25 9. A method of causing super-proliferation of a T cell *in vitro* comprising contacting a product as defined in any one of claims 1 to 3 or identified in the assay of claim 4 with a T cell.
10. A composition comprising T cells, antigen presenting cells; and a product as defined in any one of claims 1 to 3 or identified in the assay of claim 4.
- 30 11. A vaccine composition comprising an antigenic component and, as an adjuvant a product as defined in any one of claims 1 to 3 or identified in the assay of

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claim 4.

12. A pharmaceutical composition comprising a product as defined in any one of the claims 1 to 3 or identified in the assay of claim 4 and a pharmaceutically acceptable carrier or diluent.

5 13. A cell as defined in claim 6, 7 or 8, a cell made in a method according to claim 9 or a composition according to claim 10, 11 or 12 for use in a method of treatment of the human or animal body by therapy.

14. Use of a product, cell or composition as defined, made or identified in any one of claims 1 to 4 or 6 to 12 for use in the manufacture of a medicament to
10 prevent or treat cancer.

15. Use of a product as defined in any one of claims 1 to 3 or identified in the assay of claim 4 in the manufacture of a medicament to prevent or treat cancer by stimulating T cells whose T cell receptors recognise the product.

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Fig.1A.

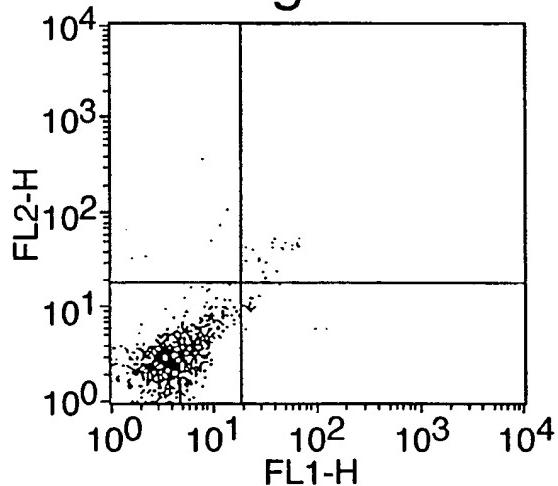


Fig.1B.

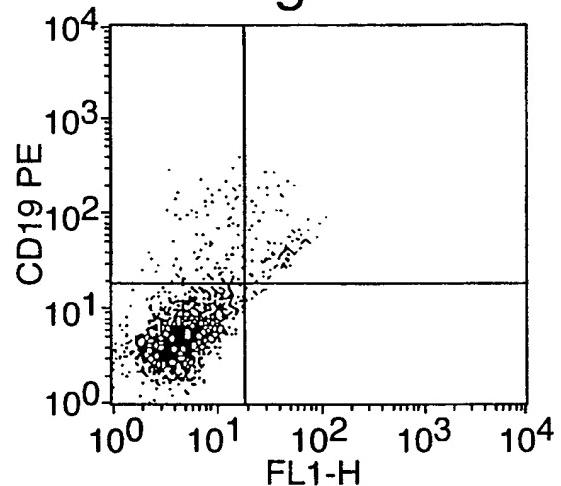
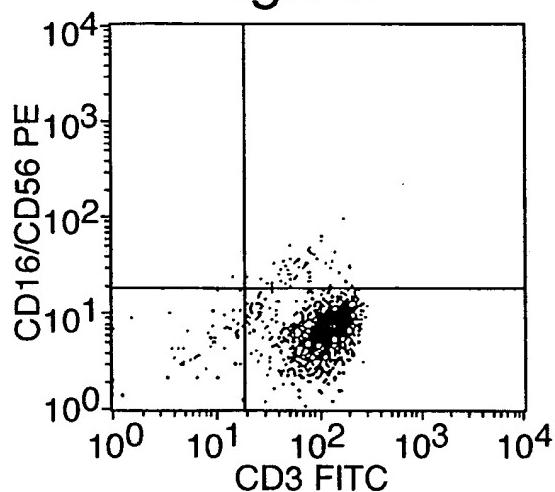


Fig.1C.



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Fig.2A.

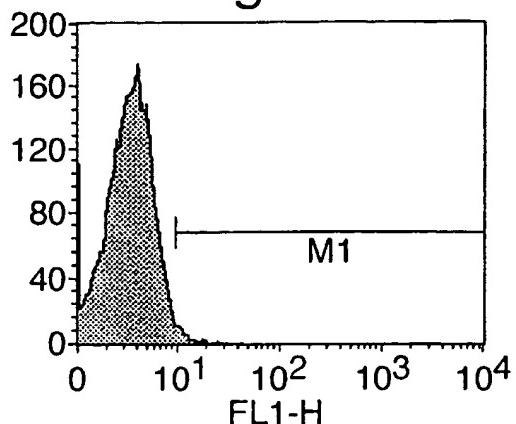


Fig.2B.

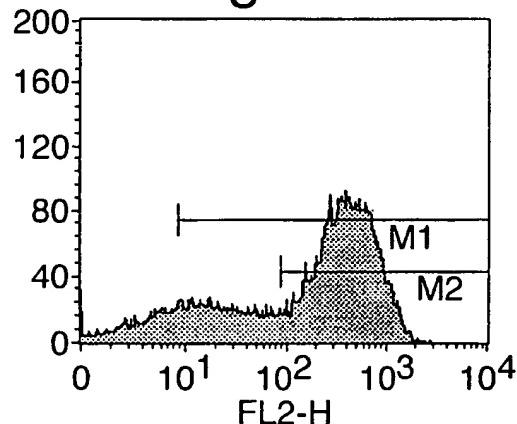


Fig.2C.

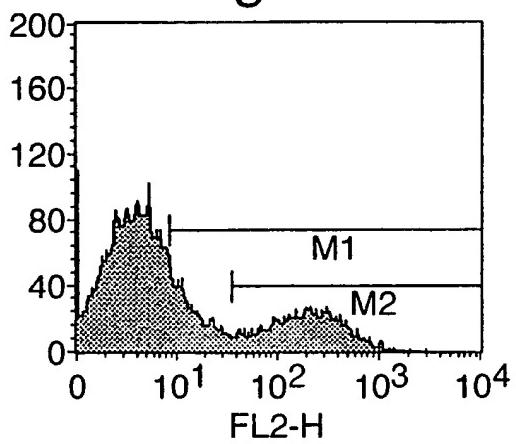


Fig.2D.

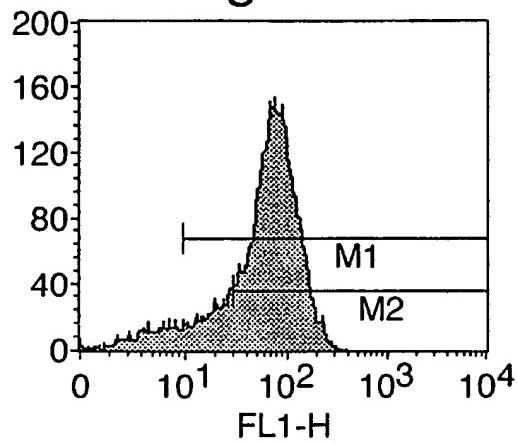
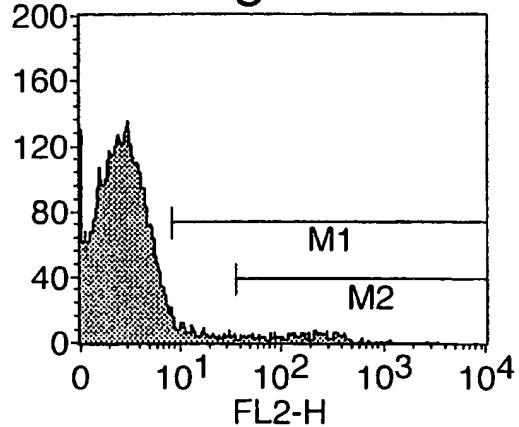


Fig.2E.



INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/00724

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/47 C12N15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, STRAND, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	DE 197 58 400 A (HANISCH FRANZ GEORG PROF DR ;MAX DELBRUECK CT FUER MOLEKULA (DE)) 1 July 1999 (1999-07-01) the whole document ---	1-15
Y	WO 96 22067 A (UNITED BIOMEDICAL INC) 25 July 1996 (1996-07-25) abstract page 36 examples claim 9 ---	1-15
Y	WO 89 08711 A (BIOMEMBRANE INST) 21 September 1989 (1989-09-21) the whole document --/-	1-15

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

^a Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
4 July 2000	12/07/2000
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epnrl, Fax: (+31-70) 340-3016	Authorized officer Panzica, G

INTERNATIONAL SEARCH REPORT

Interr.	1st Application No
PCT/GB 00/00724	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 03825 A (FINN OLIVERA J ;FONTEMOT J DARRELL (US); MONTELARO RONALD C (US)) 9 February 1995 (1995-02-09) abstract disclosure page 32 ----	1-15
A	WO 98 17300 A (DANA FARBER CANCER INST INC) 30 April 1998 (1998-04-30) abstract disclosure page 28 ----	1-15
A	WO 97 34921 A (LIVINGSTON PHILIP O ;SLOAN KETTERING INST CANCER (US); ZHANG SHENG) 25 September 1997 (1997-09-25) abstract disclosure page 11 ----	1-15
A	WO 96 07753 A (NILSSON KURT) 14 March 1996 (1996-03-14) the whole document ----	1-15
A	GB 2 288 401 A (BAY DEV CORP SA) 18 October 1995 (1995-10-18) the whole document ----	2
A	WO 92 01055 A (BOEHRINGER INGELHEIM INT) 23 January 1992 (1992-01-23) the whole document ----	2
A	EP 0 754 703 A (KIRIN BREWERY) 22 January 1997 (1997-01-22) the whole document -----	2

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 00 00724

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 3

Present claim 1 relate to an extremely large number of possible compounds. Similarly, claim 4 relates to an extremely large number of methods. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds and methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely for claim 1, those parts relating to the compounds as defined in claim 2, and for claim 4, those parts relating to the method as defined in page 7, lines 10-30.

As in regards to claim 3, the definition of its subject-matter in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the definition the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a search for claim 3 impossible.

As in regards to all the claims depending from claims 1-3, the search has been performed limitedly as depending from claim 2.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatinal Application No

PCT/GB 00/00724

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
DE 19758400	A	01-07-1999	WO	9934824 A	15-07-1999
WO 9622067	A	25-07-1996	AU	5849796 A	07-08-1996
WO 8908711	A	21-09-1989	AT	123066 T	15-06-1995
			DE	68922806 D	29-06-1995
			DE	68922806 T	29-02-1996
			EP	0357767 A	14-03-1990
			JP	11092400 A	06-04-1999
			JP	2503387 T	18-10-1990
			JP	2849683 B	20-01-1999
			US	5747048 A	05-05-1998
			US	5660834 A	26-08-1997
			US	5229289 A	20-07-1993
WO 9503825	A	09-02-1995	US	5744144 A	28-04-1998
			CA	2167730 A	09-02-1995
			EP	0804231 A	05-11-1997
			JP	9501159 T	04-02-1997
			US	5827666 A	27-10-1998
WO 9817300	A	30-04-1998	EP	0964694 A	22-12-1999
WO 9734921	A	25-09-1997	AU	2536997 A	10-10-1997
			CA	2249395 A	25-09-1997
			EP	0923605 A	23-06-1999
WO 9607753	A	14-03-1996	AU	3264195 A	27-03-1996
			EP	0779932 A	25-06-1997
			US	5882902 A	16-03-1999
GB 2288401	A	18-10-1995	NONE		
WO 9201055	A	23-01-1992	DE	4021917 A	16-01-1992
			DE	4035877 A	14-05-1992
			AT	104348 T	15-04-1994
			AU	650893 B	07-07-1994
			AU	8208291 A	04-02-1992
			CA	2084514 A	11-01-1992
			CZ	9203863 A	11-08-1993
			DE	59101397 D	19-05-1994
			DK	538300 T	10-10-1994
			EP	0538300 A	28-04-1993
			ES	2063515 T	01-01-1995
			FI	930058 A	08-01-1993
			HU	65846 A	28-07-1994
			JP	6502987 T	07-04-1994
			NO	930059 A	08-01-1993
			PL	297610 A	13-07-1992
			SK	386392 A	10-08-1994
EP 0754703	A	22-01-1997	AU	706304 B	10-06-1999
			AU	3815295 A	23-05-1996
			US	5843713 A	01-12-1998
			CN	1142229 A	05-02-1997
			WO	9613516 A	09-05-1996

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